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(57) Abstract

Methods of inhibiting tumor growth and development are described in which a combination of anti-tumor antibodies and biologically active agents, such as chemotherapeutic drugs, are utilized. The combination of anti-tumor antibody, such as the BR96 antibody, with a chemotherapeutic drug, such as doxorubicin or mitomycin C, is shown to produce a synergistic effect to inhibit tumor development and tumor cell growth.

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SYNERGISTIC THERAPY WITH COMBINATIONS OF ANTI-TUMOR ANTIBODIES AND BIOLOGICALLY ACTIVE AGENTS

FIELD OF INVENTION

The present invention relates to the use of combinations of antibody therapy and biologically active agents, such as in chemotherapy, in the treatment of disease. It is based, in part, on the surprising discovery that tumor-bearing mammals achieved significantly higher remission when treated with a combination regimen comprising treatment with anti-tumor antibody as well as chemotherapy. The methods of the invention provide a unique means for marshalling the immune system to act in concert with exogenous chemical compounds to effectively eradicate tumor cells.

BACKGROUND OF THE INVENTION

Tumor Cell Antigens and Anti-Tumor Antibodies

Tumor cells express certain antigens which are absent from, or present in small amounts on, their normal cellular counterparts. Most of these are differentiation antigens, shared by the tumor and certain embryonic cells. Some of the antigens that appear with sufficient selectivity in tumors may serve as possible targets for therapeutic

agents. This has b en r cently revi wed f r malignant melanoma, which is one of the human tumors most studied in this respect (Hellström and Hellström, in Accomplishments in Cancer Research-1984 Prize Year, General Motors Cancer Research Foundation, J.G. Fortner & J.E. Rhoads, eds., J. B. Lippincott Company, Philadelphia 1985, p. 216-240), as well as for other tumors (Burchell and Taylor-Papadimitriou, in R.W. Baldwin and V.S. Byers, eds., Monoclonal Antibodies for Tumor Detection and Drug Targeting, Academic Press, 1985, pp. 1-15; Kemshead, ibid, pp. 281-302.).

Many antibodies have been made to cell surface antigens that are expressed in greater quantities by human tumors than by normal tissues. It has also been well established that antibodies to cell surface antigens can be cytotoxic to tumor cells in the presence of complement (Hellström et al., 1962, Prog. Allergy 9:158-245), and that some antibodies can mediate antibody-dependent cellular cytotoxicity (Perlmann et al., 1969, Adv. Immunol. 11:117-193; MacLennan et al., 1969, Immunol. 17:897-910; Skurzak et al., 1972, J. Exp. Med. 135:997-1002; Pollack et al., 1972, Int. J. Cancer, 9:316-323). In the first case, an appropriate source of complement (generally rabbit or guinea pig), and in the latter case a source of effector cells (generally of mouse origin) is needed.

The evidence that antibodies to tumor-associated antigens can kill human tumor cells in the presence of human ffector cells is more recent (Hellström et al., 1981, Int. J. Cancer 27:281-285) as is the evidence that antibodies to

such antigens can kill tumor cells in the presence of human serum as a source of complement (Hellström et al., 1985, Proc. Natl. Acad. Sci. 82:1499-1502; Hellström et al., 1985, Monoclonal Antibodies and Cancer Therapy, UCLA Symposia on Molecular and Cellular Biology, Vol. 27, pp. 149-164 Alan R. Liss, Inc., NY).

Therapeutic Uses of Anti-Tumor Antibodies As Carriers of Radioisotopes, Toxins or Drugs

Attractive approaches for preparing anti-cancer agents involve labeling antibodies with radioactive isotopes (Larson et al., 1983, J. Clin. Invest. 72:2101-2114; Order, 1984, Compr. Therapy 10:9-18; Carrasquillo et al., 1984, Cancer Treatment Reports 68:317-328; de Nardo et al., 1985, Int. J. Radiation Oncology Biol. Phys. 11:335-348), or conjugating antibodies to toxins (Jansen et al., 1982, Immunol. Rev. 62:185-216; Vitetta and Uhr, 1984, Transplant. 37: 535-538) or anti-cancer drugs (Ghose et al., 1972, Brit. Med. J. 3:495-449; Hurwitz et al., 1975, Cancer Res. 35:1175-1181; Rowland et al., 1985, Cancer Immunol. Immunother. 19:1-7). The antibody gives the specificity, and the isotope or drug provides the ability to destroy the tumor. However, a disadvantage of this approach is the fact that both anti-cancer drugs and radioisotopes have a high level of toxicity to normal tissues. Thus, nonspecific uptake in various organs such as kidney, liver, or bone-marrow could lead to substantial side-effects.

SUMMARY OF THE INVENTION

The present invention relates to the use of combinations of antibody therapy with the administration of biologically active agents, such as in chemotherapy, for inhibiting the growth of tumor cells, such as in the treatment of disease by treating and inhibiting tumor development. It is based in part on observations of the surprising effectiveness of combination therapy; several tumor-bearing mammals who had received the anti-tumor antibody BR96 achieved significantly greater inhibition of tumor growth in response to chemotherapy. Similar tumor bearing mammals exhibited a significantly lower level of response to chemotherapy alone and did not respond at all to the antibody alone the way it was given.

In particular embodiments of the invention, an anti-tumor antibody such as, preferably, BR96 monoclonal antibody is administered to mammals who are subsequently or concurrently treated with standard chemotherapy regimens. In preferred embodiments of the invention, chemotherapy is administered concurrent with antibody treatment. It is suggested that the effectiveness of combination therapy can be attributable to antibodies at the tumor site which render the malignant cells more susceptible to the toxic effects of chemotherapeutic agents or induce an immune response in a mammal that synergizes with the chemotherapy drugs.

DESCRIPTION OF THE DRAWINGS

In the drawings:

FIGURE 1 illustrates the affect on tumor volume of treating nude mice (Balb/c nu/nu females) with BR96 antibody, or doxorubicin (Adr) and with combination of BR96 and Adr. Human lung adenocarcinoma (H2707) tumor implants were inserted into the right rear flank of each mouse and the mice were separated into treatment groups (8 mice/group). Control mice (open circles) received PBS injections; the remaining mice were treated with Adr alone (7 mg/kg/injection, closed squares), BR96 alone (closed circles); BR96 together with 5 mg/kg/injection of Adr (open triangles) and BR96 with 7 mg/kg/injection of Adr (closed triangles).

FIGURE 2 illustrates the inhibition of tumor volume in nude mice treated with BR96 antibody and mitomycin C (MMC). Human lung adenocarcinoma H2707 tumor implants were inserted into the right rear flank of Balb/c nu/nu female mice, and the mice were divided into treatment groups. Control mice (open circles) received PBS injection; the remaining mice received MMC alone (3 mg/kg/injection, closed squares), BR96 alone (closed circles); BR96 and 2 mg/kg/injection MMC (open triangles) and BR96 and 3 mg/kg/injection MMC (closed triangles).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to therapeutic regimens comprising treatment with anti-tumor antibodies and standard chemotherapy. In preferred embodiments of the invention, the anti-tumor antibodies react with antigens on the surface of tumor cells. In a most preferred embodiment the anti-tumor antibody is the monoclonal antibody BR96.

Although there is no duty to explain the efficacy of antibody/chemotherapy regimens in tumor cell killing, we speculate that binding to the surface of tumor cells, antibodies, which like BR96 are internalizing, may render cells more susceptible to chemotherapeutic killing, possibly by increasing the drug uptake. When given to an immunocompetent individual, rather than a nude mouse, treatment with anti-tumor antibody may offer further benefit by inducing an immune response which synergizes with chemotherapy drugs, such as doxorubicin (adriamycin) and mitomycin C, either by making the cells more sensitive to the drugs or by making the cells more sensitive to an animal's or patient's immune response.

For purposes of clarity of disclosure, and not by way of limitation, the detailed description of the invention will be divided into the following subsections:

(i) characteristics of the antibody molecules of the inventi n;

(ii) preparation of monoclonal antibodies; and

(iii) tumor therapy with combinations of anti-tumor antibodies and biologically active agents.

Characteristics of the Antibody Molecules of the Invention

Antibodies of virtually any origin can be used according to the present invention, but in preferred embodiments the antibodies define a tumor-associated antigen. Monoclonal antibodies offer the advantage of a continuous, ample supply. In fact, by immunizing mice with tumor-associated antigens, and establishing hybridomas making antibodies to such antigens, it should be possible to rapidly establish a panel of antibodies capable of reacting with and treating a large variety of tumors.

The BR96 antibody is of the IgG3 subclass. The antibody displays a high specificity for carcinoma cells of different organ types, for example, tumors of the breast, lung, colon and ovary as well as cultured cell lines established from various breast, lung and colon carcinomas. Furthermore, the BR96 antibody shows no binding to other types of tumor cells such as the T-cell lymphoma cells lines, CEM and MOLT-4, the B cell lymphoma cell line P3HR-1 or melanoma cell lines. The BR96 antibody is able to be internalized in antigen-positive tumor cells, as shown, for example, by election microscopy, it is toxic on antigen-positive tumor cells, mediates ADCC and CDC activity, and surprisingly, it is cytotoxic alone, i.e. in unmodified form

when applied at a sufficently high d se. The BR96 antibodies appear to recognize a fucosylated Le^y antigen, or an antigen closely related to such an entity.

The term "BR96 antibody" as used herein includes whole, intact polyclonal and monoclonal antibody molecules such as the murine BR96 monoclonal antibody produced by hybridoma ATCC No. HB10036, and chimeric antibody molecules such as chimeric BR96 antibody produced by hybridoma ATCC No. HB10460. The BR96 antibody described above includes any fragments thereof containing the active antigen-binding region of the antibody such as Fab, $F(ab')_2$ and Fv fragments, using techniques well established in the art [see, e.g., Rouseauz et al., "Optimal Conditions For The Preparation of Proteolytic Fragments From Monoclonal IgG of Different Rat IgG Subclasses", in Methods Enzymol., 121:663-669 (Academic Press 1986)]. The BR96 antibody of the invention also includes fusion proteins.

In addition, the BR96 antibody does not display any immunohistologically detectable binding to normal human tissues from major organs, such as kidney, spleen, liver, skin, lung, breast, colon, brain, thyroid, heart, lymph nodes or ovary. Nor does the antibody react with peripheral blood leukocytes. BR96 antibody displays limited binding to some cells in the tonsils and testes, and binds to acinar cells in the pancreas, and to epithelial cells in the stomach and esophagus. Thus, the BR96 antibody is superior to m st known anti-tumor antibodies in the high d gree of specificity for tumor cells as compared to n rmal cells

[see, .g., Hellström t al., "Immunological Approaches To Tumor Therapy: Monoclonal Antibodies, Tumor Vaccines, And Anti-Idiotypes", in Covalently Modified Antigens And Antibodies In Diagnosis And Therapy, Quash/Rodwell (eds.), pp. 1-39 (Marcell Dekker, Inc., 1989) and Bagshawe, "Tumour Markers - Where Do We Go From Here", Br. J. Cancer, 48:167-175 (1983)].

Preparation of Monoclonal Antibodies

According to the invention, monoclonal antibodies can be produced using any method known in the art, including but not limited to the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497) as well as the trioma technique, the human B-cell hybridoma technique (Kozborn et al., 1983, Immunology Today 4:72), the EBV-hybridoma technique (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. pp. 77-96, and Huse et al., 1989, Science 246:1275-1281), as well the chimeric antibody techniques discussed infra.

While the invention is demonstrated using mouse monoclonal antibodies, the invention is not so limited; in fact, human antibodies can be used and may prove to be preferable. Such antibodies can be obtained by using human hybridomas (Cote et al., 1983, Proc. Natl. Acad. Sci., 80:2026-2030) or by transforming human B cells with EBV virus in vitro (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, pp. 77-96). In fact,

according to the invention, techniques wer d veloped for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al, 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity.

The subsections below describe how the antibody used in the examples which follow was prepared.

A monoclonal antibody of this invention, designated BR96, was produced via the hybridoma techniques described hereinbelow using a breast cancer cell line H3396 as the immunogen. The BR96 hybridoma, prepared as described hereinbelow and producing the BR96 antibody, was deposited on February 22, 1989 with the ATCC, and has there been identified as follows:

BR96 ATCC Accession No.: HB 10036

According to another embodiment, $F(ab')_2$ fragments of the BR96 monoclonal antibody were produced by pepsin digestion of purified BR96 [Nisonoff et al., "The Antibody Molecule", Academic Press, New York (1975)], as described hereinbelow. The binding of the $F(ab')_2$ fragments to tumor (H3396) and MCF7 cells was shown to be comparable to the binding of the whole BR96 monoclonal antibody.

Additionally, a chimeric (murine/human) antibody was produced using a two-step homolog us recombinati n procedure as described by F ll et al., in Proc. Natl. Acad.

Sci. USA 86:8507-8511 (1989) and in co-pending patent applications U.S. Serial Number 243,873, filed September 14, 1988, and Serial Number 468,035, filed June 22, 1990, assigned to the same assignee as the present application; the disclosures of all these documents are incorporated in their entirety by reference herein. This two-step protocol involves use of a target vector encoding human IgGgamma1 heavy chain to transfect a mouse hybridoma cell line expressing murine BR96 monoclonal antibody (hybridoma ATCC No. HB 10036) to produce a hybridoma expressing a BR96 chimeric antibody containing human IgGgamma1 heavy chain. This hybridoma is then transfected with a target vector containing DNA encoding human kappa (K) light chain to produce a murine hybridoma expressing a BR96 chimeric antibody containing human K light chain. The target vectors used to transfect the hybridomas are the pHgamma1HC-DD_4 vector digested with Xba1 enzyme (Oncogen, Seattle, WA) and the HindIII digested pSV_2gpt/C_k vector (Oncogen, Seattle, WA).

The chimeric BR96 hybridoma, identified herein as ChiBR96, prepared as described hereinbelow and producing th chimeric human/murine BR96 antibody, was deposited on May 23, 1990, with the ATCC, and has there been identified as follows:

ChiBR96 ATCC Accession No.: HB 10460

Once the hybridoma that expresses the chimeric antibody is identifi d, th hybridoma is cultured and the desir d chimeric mol cules ar isolated from th cell

cultur supernatant using techniques well known in the art for isolating monoclonal antibodies.

In addition, the present invention encompasses antibodies that are capable of binding to the same antigenic determinant as the BR96 antibodies and competing with the antibodies for binding at that site. These include antibodies having the same antigenic specificity as the BR96 antibodies but differing in species origin, isotope, binding affinity or biological functions (e.g., cytotoxicity). For example, class, isotope and other variants of the antibodi s of the invention having the antigen-binding region of the BR96 antibody can be constructed using recombinant class-switching and fusion techniques known in the art [see, e.g., Thammana et al., "Immunoglobulin Heavy Chain Class Switch From IgM to IgG In A Hybridoma", Eur. J. Immunol., 13:614 (1983); Spira et al., "The Identification of Monoclonal Class Switch Variants By Subselection And ELISA Assay". J. Immunol. Meth., 74:214-221 (1986)]. Thus, other chimeric antibodies or other recombinant antibodies (e.g., fusion proteins wherein the antibody is combined with a second protein such as a lymphokine or a tumor inhibitory growth factor) having the same binding specificity as the BR96 antibodies fall within the scope of this invention.

Tumor Therapy with Combinations of Anti-Tumor Antibodies and Biologically Active Agents

The present invention provides for combination therapy comprising treatment with anti-tumor antibody as well as treatment with a biologically active agent, such as in a standard chemotherapy regimen. In preferred embodiments of the invention, chemotherapy is administered concurrently with antibody therapy.

The antibodies utilized in the invention are anti-tumor antibodies, preferably monoclonal antibody BR96. In particular embodiments of the invention, it is desirable to utilize whole antibody molecules, whereas in alternative embodiments it will be desirable to use fragments of antibody molecules including but not limited to Fv, F(ab) and $F(ab')_2$ fragments. Such fragments can bind to tumor cells and render said cells more susceptible to chemotherapeutic agents while minimizing immune functions related to the Fc region of the antibody molecule and minimizing the generation of an immune response directed at heterologous Fc region. Alternatively, it may be desirable to engineer monoclonal antibodies to comprise human Fc regions so as to maximize immune functions related to the Fc region. Accordingly, the present invention permits tailoring antibody therapy to better conform to specific situations.

The ch motherapeutic r gimens utilized according to the invention include any regimen believed to be suitable

for the treatment of the tumor r malignancy. Different malignancies can require the use of specific anti-tumor antibodies and specific chemotherapy regimens, which will be determined on a case by case basis. The present invention relates to any malignant condition, including, but not limited to adenocarcinomas such as breast carcinoma and colon carcinoma, non-small cell lung carcinoma, leukemia, lymphoma and neuroectoderm derived tumors including melanoma, astrocytoma and glioblastoma.

The use of anti-tumor antibody therapy and chemotherapy combination treatment is exemplified in the Examples that follow. According to the invention it is desirable to ensure that the anti-tumor antibody is capable of contacting its tumor cell target. Therefore, in mammals bearing tumors which are relatively inaccessible to exogenously administered antibodies, including brain tumors, it can be desirable to either administer antibodies locally into the tumor or, in the case of brain tumors, to render the blood brain barrier more permeable, for example with an osmotic agent, or to administer antibody or antibody fragments into the cerebrospinal fluid or via the carotid artery.

Having generally described this invention, a further understanding can be obtained by reference to certain specific examples which are provided herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

EXAMPLE 1

Inhibition of Tumor Development in Nude Mice

Nude mice (Balb/c nu/nu females) were segregated into eight specific treatment groups (8 mice/group). Each group received an implant of an approximately 3mm x 3mm piece of an H2707 tumor (establised from a metastasis of a human lung adenocarcinoma which had been established in culture at Oncogen); the tumor pieces were inserted into th right rear flank. Tumors grew and developed in all mice.

On Days 13, 17 and 21 following tumor implantation, the mice were administered a specific treatment regimen based upon their grouping.

The Control group of mice received an injection of phosphate buffered saline (PBS, 0.2 ml). The remaining groups of mice received injections of BR96 (0.5 mg/injection in 0.2 ml of PBS) or a chemotherapeutic drug or a combination of both. Chemotherapeutic drugs were administered in a volume of approximately 0.2 ml; doxorubicin (adriamycin) was used at a dosage of either 5 mg/kg/injection or 7mg/kg/injection, and mitomycin C was administered at 2 mg/kg/injection and 3 mg/kg/injection. All treatments were administered on Days 13, 17 and 21 after tumor implant. Tumor volumes were determined on Days 13, 20, 26, 36, 43 and 50 post-implant. The results of this study are shown in Figures 1 and 2.

At a dosag f 7 mg/kg/injecti n adriamycin was toxic to the mice, killing all eight mice in the group by day 50. When BR96 was administered together with the 7 mg/kg/injection dosage of adriamycin, all of the mice in that group were dead by day 42.

Reducing the dosage of adriamycin to 5 mg/ml/injection continued to produce some toxicity in combination with BR96 resulting in two mice of the eight in the group dying by day 42.

Administration of mitomycin C (3 mg/kg/injection) produced a significant reduction in tumor volume when given in conjunction with BR96 administration. Mitomycin C (3 mg/kg/injection) administration alone inhibited tumor growth, but not to the same extent as found for the combination BR96/mitomycin C treatment.

When the dosage of mitomycin C was reduced to 2 mg/kg/injection and administered with BR96, a small increase in anti-tumor activity was found over that seen for the administration of BR96 alone. No toxic effects due to mitomycin C were seen in any animals at the dosages utilized.

Although BR96 has an anti-tumor activity by itself, as previously demonstrated, no anti-tumor activity of the monoclonal antibody has been detected when given to mice at the dosage and the time-points used in the present study.

EXAMPLE 2

Effect Of Combinations Of

Doxorubicin And BR96 Upon Growth Of Tumor Cells

Human breast carcinoma cells (H3396 and H3630) established as lines in tissue culture at Oncogen, were plated into wells of 96-well flat bottom plates at a density of 10^4 cells/well in 100 µl of IMDM containing 10% fetal calf serum. The plates were maintained at 37°C for 12 to 16 hours in order to allow the cells to become adherent to th wells. The medium was then removed from each well and replaced with either 100 µl of fresh medium or 100 µl of medium containing BR96 and/or doxorubicin (adriamycin). Various concentrations of BR96 and/or doxorubicin were studied. The cells were then maintained at 37°C for 18 hours, at which time 1 microcurie (1µCi) of [^{3}H]-thymidine (^{3}H-TdR) was added to each well, and the cells maintained for an additional 6 hours at 37°C. The plates were then frozen for 6 hours at -20°C, thawed and each well was harvested onto a glass fiber filter, washed and radioactivity corresponding to DNA synthesis determined by scintillation counting. The results, shown in Table I, illustrate the synergistic effect of BR96 and doxorubicin (Adr) at low concentrations for each breast cancer cell lin tested. In the absence of BR96, higher (more toxic) concentrations of Adr are necessary to inhibit cell growth. In the absence of Adr, high conc ntrati ns f BR96 are

required to produce any significant inhibition of cell growth.

TABLE 1

H3396

Adr Concentration (μM)	% Inhibition @ BR96 Concentrations 0 μg/ml	1 μg/ml	10 μg/ml	100 μg/ml	1000 μg/ml
0.0000	0%	-33%	0%	24%	48%
0.0025	-17%	-14%	21%	39%	56%
0.0100	-3%	-13%	23%	38%	58%
0.0400	15%	-8%	29%	40%	54%

H3630

Adr Concentration (μM)	% Inhibition @ BR96 Concentrations 0 μg/ml	1 μg/ml	10 μg/ml	100 μg/ml	1000 μg/ml
0.0000	0%	-27%	-7%	15%	44%
0.0025	-8%	-1%	15%	24%	49%
0.0100	3%	0%	14%	26%	52%
0.0400	15%	7%	17%	29%	58%

The combination of BR96 and Adr produces significant cell growth inhibition at lower concentrations of BR96 and

Adr; the anti-tumor effect for the combined treatment is significantly greater than the additive effect of BR96 and Adr at these low concentrations.

The foregoing description and Examples are intended as illustrative of the present invention, but not as limiting. Numerous variations and modifications may be effected without departing from the true spirit and scope of the present invention.

We Claim:

1. A method of inhibiting the growth of tumor cells comprising contacting said tumor cells with an antibody that binds to the cells and a biologically active agent capable of modifying the proliferation of said cells.

2. The method according to Claim 1, wherein said biologically active agent is a chemotherapeutic drug.

3. The method according to Claim 2, wherein said chemotherapeutic drug is selected from the group consisting of doxorubicin and mitomycin C.

4. The method according to Claim 1, wherein said antibody is a monoclonal antibody having a substantially similar binding specificity as BR96 as deposited with the ATCC having accession number HB 10036.

5. The method according to Claim 1, wherein said tumor cells are human tumor cells.

6. The method according to Claim 1, wherein said tumor cells are contacted in vivo in a mammal with said antibody and said biologically active agent.

7. A method of tr ating tumor d vel pment in a mammal comprising administering to said mammal an antibody

that binds to cells of said tumor and a bi logically active agent capable for modifying the proliferation of said tumor cells.

8. The method according to Claim 7, wherein said biologically active agent is a chemotherapeutic drug.

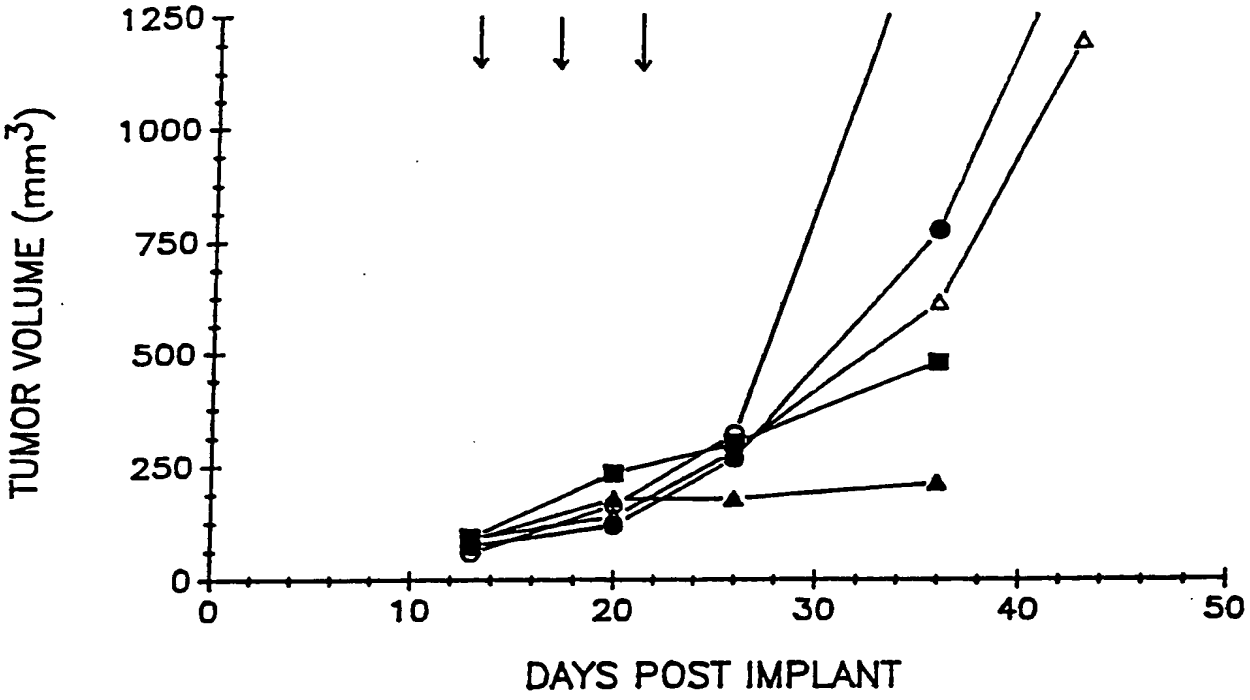
9. The method according to Claim 8, wherein said chemotherapeutic drug is selected from the group consisting of doxorubicin and mitomycin C.

10. The method of Claim 7, wherein said antibody is a monoclonal antibody having a substantially similar binding specificity as BR96 as deposited with the ATCC having accession number HB 10036.

11. The method of Claim 7, wherein said tumor cells are lung adenocarcinoma cells.

12. The method of Claim 7, wherein said tumor cells are breast cancer cells.

Figure 1


1250
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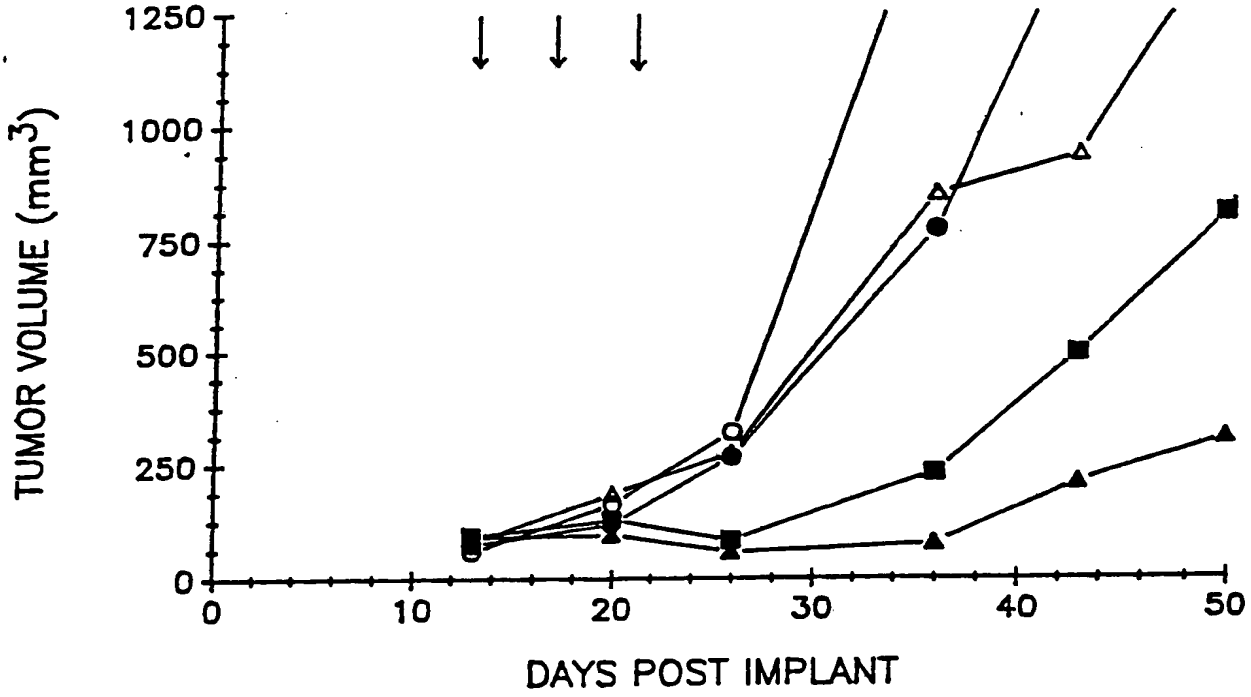
500
250
0
TUMOR VOLUME (mm3)
0
10
20
30
40
50
DAYS POST IMPLANT

Figure 2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1000
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500
250
0
0
10
20
30
40
50
DAYS POST IMPLANT